fenamic acid while acetylsalicylic acid, sodium salicylate, phenylbutazone, and oxypenbutazone were unaffected. In the presence of 5% ethanol, the protective action of acetylsalicylic acid was attenuated, while that of indomethacin, mephenamic acid, or phenylbutazone was unaltered. Although 1% DMSO or 5% ethanol did not stabilize erythrocytes to heat-induced hemolysis per se, 5, 10, and 20% DMSO did so, while 10% ethanol enhanced hemolysis.

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Received Dec. 1, 1967. P.S.E.B.M., 1968, Vol. 128.

Mechanism of Norepinephrine Depletion by 5-Hydroxytryptophan (33052)

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DL-5-Hydroxytryptophan (5-HTP) at high doses produces overt stimulation in the rat characterized by piloerection, panting, and increased motor activity including circling and backing up. Brodie *et al.*, have suggested that excitation produced by 5-HTP is associated with release of brain norepinephrine (NE) (1). Their data demonstrated that a large dose of 5-HTP reduced rat brain NE by 50%.

The decarboxylation of 5-HTP to serotonin (5-HT) is catalyzed by the same enzyme, L-aromatic amino acid decarboxylase, which converts dihydroxyphenylalanine (DOPA) to dopamine (DA) in an intermediate step in the synthesis of NE (2). This large dose of 5-HTP together with its efficiency for penetrating the CNS might be expected to produce substrate inhibition of the decarboxylase and thereby inhibit decarboxylation of DOPA.

This report confirms the effect of 5-HTP in lowering rat brain NE. However, data presented here indicate that inhibition of NE synthesis rather than a 5-HTP (or 5-HT) mediated release is the cause of NE depletion.

Methods and Materials. L-α-methyltyrosine (L-α-MT) was obtained from Merck Institute for Therapeutic Research, DL-α-methyltyrosine methyl ester (H 44/68) from Dr. H. Corrodi, Hassle Laboratories, and pargyline from Abbott Laboratories. All other compounds are commercially available.

Upjohn Sprague-Dawley male rats (125–150 gm) were used in this investigation. Where fasted conditions are noted rats were deprived of food overnight (16–20 hours). Fasted, weanling rats (23 days old) were used in the incorporation experiment.

The i.v. administration of H 44/68 was facilitated by dissolving the drug in Merlis solution (3). Except for a solubilized preparation of reserpine, all other compounds were suspended in 0.25% aqueous methylcellulose and administered i.p. In each experiment control rats also received the appropriate diluent.

In brain amine studies rats were sacrificed by decapitation, brains were removed and

TABLE I. Effect of DL-5-HTP (600 mg/kg, i.p.) upon Rat Brain Norepinephrine Levels.[a]

Condition	NE (μg/gm)
Fasted control	0.29 ± 0.01
5-HTP-treated	0.15 ± 0.01
Nonfasted control	0.27 ± 0.01
5-HTP-treated	0.17 ± 0.01

[a] Rats were sacrificed 2 hours after drug administration. Each value is the average of three determinations ± SEM.

placed on dry ice. Individual whole rat brains were homogenized and NE was extracted (4) and assayed spectrophotofluorometrically (5). All NE values are expressed as μg/gm of wet weight of brain tissue ± SEM.

In the incorporation experiment 5-HTP (600 mg/kg) was administered 1 hour before 50 μC L-tyrosine-^{14}C u.l., specific activity > 350 mC/mmol, New England Nuclear Corp.). The extent of incorporation of precursor into rat brain DA and NE 1 hour after its i.v. administration was determined as previously reported (6).

The ability of various compounds to alter the initial decline in rat brain NE levels after inhibition of its synthesis with H 44/68 (200 mg/kg, i.v.) or α-MT (200 mg/kg, i.p.) was determined. Compounds were administered 5 min after H 44/68 or α-MT and rats were sacrificed 1 hour after receiving the inhibitor. Brain NE was determined as described above.

Results. Reproducible stimulation in rats was obtained with a single 600 mg/kg i.p. dose of 5-HTP. Data from a single experiment (Table I) confirm the decrease in rat brain NE after 5-HTP noted by Brodie *et al.* (1). Depletion was marked in both fed and fasted rats.

Effect of pretreatment of rats with 5-HTP on incorporation of tyrosine-^{14}C into rat brain DA and NE is shown in Table II. The 5-HTP markedly decreased the levels of label in both DA (35% of control) and NE (28% of control). Total brain NE was reduced to 45% of control levels. The reduced specific activity of brain NE from treated rats indicates that although synthesis of NE was being achieved as indicated by the presence of NE-^{14}C, the rate of synthesis was well below that detected in control animals.

Synthesis of NE is blocked by the competitive inhibitor of tyrosine hydroxylase, α-MT (7) and also by its methyl ester, H 44/68 (8). After administration of α-MT in sufficient quantities to effectively inhibit NE synthesis, tissue catecholamine levels decline at their rate of catabolic decay (9). Since tyrosine hydroxylase is the rate-limiting step in catecholamine biosynthesis, further treatment with a second inhibitor of this enzyme or concurrent inhibition of L-aromatic amino acid decarboxylase should not alter the rate of NE utilization or the rate of decline of tissue NE levels. Data on effect of 5-HTP on the NE levels in rat brain after H

TABLE II. Incorporation of L-Tyrosine-^{14}C into Rat Brain Norepinephrine after DL-5-HTP.[a]

Condition	No. of rats	DA (cpm)	NE (cpm)	NE (μg/gm)	NE (sp. act.[b])
Control	2	5430	1460	0.37	530
		4000	1160	0.28	500
	Av	4720	1310	0.33	520 ± 20
DL-5-HTP (600 mg/kg)	4	1210	270	0.13	240
		1360	310	0.15	250
		1960	390	0.14	310
		2130	500	0.19	360
	Av	1670	370	0.15	290 ± 20

[a] See "Methods and Materials" for dosing regimen.
[b] Specific activity is cpm/mμmol of NE and is the average of the individual values.

TABLE III. Effect of DL-5-HTP on Depletion of Rat Brain NE after α-MT: Comparison with *d*-Amphetamine, Pargyline, and Reserpine.[a,b]

	Condition	No. of animals	NE (μg/gm)
I.	Control	4	0.33 ± 0.01
	H 44/68 (200) i.v.	4	0.24 ± 0.01
	5-HTP (600) i.p.	3	0.26 ± 0.01
	H 44/68 (200) i.v. + 5-HTP (600) i.p.	4	0.23 ± 0.01
II.	Control	5	0.35 ± 0.01
	H 44/68 (200) i.v.	6	0.25 ± 0.01
	H 44/68 (200) i.v. + *d*-amphetamine sulfate (1) i.p.	3	0.17 ± 0.01
	H 44/68 (200) i.v. + pargyline • HCl (100) i.p.	3	0.29 ± 0.01
III.	Control	3	0.35 ± 0.01
	α-MT (200) i.p.	3	0.26 ± 0.01
	α-MT (200) i.p. + reserpine (2.5) i.p.	3	0.15 ± 0.02

[a] Dosing procedure described in "Materials and Methods."
[b] Dose of drug in mg/kg is given in parentheses.

44/68 are compared with data from other experiments in which reserpine, *d*-amphetamine, and pargyline were used (Table III). The 5-HTP produced no change in rate of NE decay under these conditions. Both *d*-amphetamine and reserpine, which have been reported to release brain NE, accelerated the decline in brain NE levels after H 44/68 or α-MT. Pargyline, by antagonizing the catabolism of NE by monoamine oxidase, slowed the initial rate of decay of brain NE.

Discussion. The i.p. dose of 5-HTP required to produce excitation in rats described by Brodie *et al.* was 600 mg/kg. Although the overt behavior produced by this large dose varied to some extent with fasting conditions, 5-HTP did effectively lower brain NE in both fasted and fed rats. We did not determine brain NE levels after administration of 5-HTP in quantities which did not produce characteristic stimulation. In support of the concept of Brodie *et al.* that excitation produced by large doses of 5-HTP is due to a release of NE, is the report by Carlsson *et al.* that 5-HTP did not elicit this effect in mice depleted of brain NE by extremely high doses of reserpine (20–40 mg/kg) (10).

An alternative to the release mechanism for depletion of NE is the suggestion that 5-HTP at these doses inhibits the synthesis of brain NE. The 5-HTP and DOPA each inhibit competitively the decarboxylation of the other by L-aromatic amino acid decarboxylase (11). *In vivo* inhibition of guinea pig heart tyrosine hydroxylase by 5-HTP (doses not specified) (12) suggests also a possible inhibition of the rate-limiting step in brain NE biosynthesis by this massive dose of 5-HTP. A dual inhibitory effect of 5-HTP on both these enzymes would reduce NE, but likely at a rate no faster than the extent to which tyrosine hydroxylase is blocked.

Both H 44/68 and α-MT decreased brain NE by approximately 0.10 μg/gm in the first hour indicating a half-life of slightly less than 2 hours for rat brain NE (Table III). At no time did we observe a decrease in brain NE after 5-HTP that exceeded the rate of NE depletion after H 22/54. Decrease in NE after 5-HTP in Table III equaled that observed with H 44/68. Compounds which are known to release NE, such as reserpine and *d*-amphetamine, successfully increased the depletion of NE to 0.20 μg/gm and 0.18 μg/gm in 1 hour. As expected pargyline significantly slowed the rate of NE depletion in the first hour.

Diminished levels of incorporation in both DA and NE isolated after L-tyrosine-^{14}C administration (Table III) support the concept of *in vivo* inhibition of catecholamine biosynthesis by 5-HTP. The diminished value for the specific activity of the NE remain-

ing after 5-HTP further sustantiates this inhibition of synthesis concept as the mechanism of 5-HTP induced NE depletion.

Summary. The 5-HTP (600 mg/kg, i.p.) reduced rat brain NE to 70% of control levels after 2 hours. The decrease in brain NE after large doses of 5-HTP may be due to inhibition of the biosynthesis of this biogenic amine. In support of this suggestion 5-HTP reduced both incorporation of tyrosine-^{14}C into rat brain NE, and specific activity of the isolated NE. Data are also presented to show that the decrease in brain NE is not due to release such as that produced by reserpine or *d*-amphetamine.

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Received Dec. 8, 1967. P.S.E.B.M., 1968, Vol. 128.

Ascorbic Acid Deficiency in the Squirrel Monkey* (33053)

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Evidence that primates require exogenous ascorbic acid has not been established for all species. The general statement that monkeys require exogenous vitamin C is based in large part on evidence for such a need in the rhesus monkey, *Macaca mulatta* (1). A requirement for exogenous ascorbic acid has also been reported for *Cebus fatuellus* (2), *Macaca cyclopsis swinhoi* (1), and *Cercopithecus aethiops* (3). Elliot *et al.* (4) reported that liver slices of the prosimian primates, *Tupaia glis* and *Nycticebus coucang*, can synthesize ascorbic acid from gulonolactone *in vitro*, and presumably do not require an exogenous source of this vitamin. Chatterjee *et al.* (5), however, have noted that D-glucurono reductase, which converts D-glucuronolactone to L-gulonolactone, is specifically absent in those species which cannot synthesize L-ascorbic acid. If these prosimians can make the conversion of D-glucuronolactone to L-gulonolactone they presumably would not require an exogenous source of this vitamin. The present study was done to determine whether the squirrel monkey, *Saimiri sciureus*, requires an exogenous source of ascorbic acid.

Materials and Methods. Six juvenile Brazilian squirrel monkeys, obtained from Leticia, Colombia were used in this study. The monkeys were fed a diet devoid of ascorbic acid (Table I). After 3 months on the ascorbic acid free regimen, 3 of the animals were supplemented with ascorbic acid at a level of 10 mg/kg of body weight per day. The ascorbic acid was given intramuscularly as an aqueous solution, prepared just prior to administration. The remainder of the animals were continued on the ascorbic acid free regimen for the 4-month period of study. Body weight, packed cell volume, and serum ascor-

* Supported by grant FR-00180 from the National Institutes of Health.